ab186035 – SMAD1 (pS463/S465) + SMAD1 Total SimpleStep ELISA® Kit

For the semi-quantitative measurement of SMAD1 (pS463/S465) and SMAD1 Total in human and mouse cell lysates.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab186035

Storage and Stability: Store kit at 2-8ºC immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Standard Preparation and Reagent preparation sections.

Materials Supplied

Item	Quantity	Storage Condition
SMAD1(pS463/S465) Capture Antibody	1.5 mL	+4ºC
SMAD1(pS463/S465) Detector Antibody	1.5 mL	+4ºC
SMAD1Total Capture Antibody	1.5 mL	+4ºC
SMAD1Total Detector Antibody	1.5 mL	+4ºC
Control Lysate*	1 Vial	+4ºC
10X Wash Buffer PT	15 mL	+4ºC
5X Cell Extraction Buffer PTR	12 mL	+4ºC
50X Cell Extraction Enhancer Solution	1 mL	+4ºC
TMB Substrate	12 mL	+4ºC
Stop Solution	12 mL	+4ºC
Pre-Coated 96 Well Microplate (12 x 8 well strips)	96 Wells	+4ºC
Plate Seal	1	+4ºC

***** Prepared from C2C12 cells, cultured to confluence in T175 flasks in 10% FBS containing medium, then treated with 50ng/mL rh BMP4 for 30min

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

Microplate reader capable of measuring absorbance at 450 or 600 nm.
Method for determining protein concentration (BCA assay recommended).
Deionized water.
Multi- and single-channel pipettes.
Tubes for standard dilution.
Plate shaker for all incubation steps.
Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).
PBS.

Reagent Preparation

Equilibrate all reagents to room temperature (18-25ºC) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.
Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations

1X Cell Extraction Buffer PTR (For cell and tissue extracts only): Prepare 1X Cell Extraction Buffer PTR by diluting 5X Cell Extraction Buffer PTR and 50X Cell Extraction Enhancer Solution to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 7.8 mL deionized water, 2 mL 5X Cell Extraction Buffer PTR and 200 µL 50X Cell Extraction Enhancer Solution. Mix thoroughly and gently. If required protease inhibitors can be added.
Alternative – Enhancer may be added to 1X Cell Extraction Buffer PTR after extraction of cells or tissue. Refer to note in the Troubleshooting section.
1X Wash Buffer PT: Prepare 1X Wash Buffer PT by diluting 10X Wash Buffer PT with deionized water. To make 60 mL 1X Wash Buffer PT combine 6 mL 10X Wash Buffer PT with 54 mL deionized water. Mix thoroughly and gently.
5X Cell Extraction/Enhancer Buffer PTR: Prepare 5X Cell Extraction/Enhancer Buffer PTR by adding 1/10th volume of 50X Cell Extraction Enhancer Solution. To prepare 1 mL, add 100 µL of 50X Cell Extraction Enhancer Solution to 900 µL of 5X Cell Extraction Buffer PTR. This concentrated mix is used for lysing cells directly in cell culture medium.
Antibody Cocktail: Prepare Antibody Cocktail by combining an appropriate volume of the capture and detector antibodies immediately prior to assay. To make 3 mL of the Antibody Cocktail combine 1.5 mL Capture Antibody with 1.5 mL Detector Antibody. Mix thoroughly and gently.

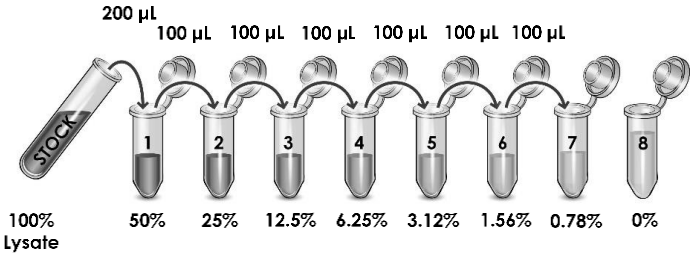
Control Lysate Preparation

Kit Control lysates are provided at a concentration that give consistent signal between different lots. Lysates are produced and formulated by signal intensity to be consistent to within 30% of the previous lot. As such, Control lysates are not provided with a protein concentration.
Always prepare a fresh set of positive controls for every use. Prepare serially diluted control lysates immediately prior to use. Discard working lysate dilutions after use as they do not store well. The following section describes the preparation of a lysate dilution series for duplicate measurements (recommended).

Reconstitute the Lyophilized SMAD1 Control Lysate* by adding 250 µL water. Mix thoroughly and gently. Hold at room temperature for 1 minute and mix gently. This is the 100% **Stock Lysate** Solution. Remaining stock material should be aliquoted and stored at -80 °C.
Label eight tubes, Controls 1– 8.
Add 200 µL of 1X Cell Extraction Buffer PTR into tube number 1 and 100 µL of 1X Cell Extraction Buffer PTR into numbers 2-8.
Use the Stock Lysate to prepare the following dilution series. Control #8 contains no protein and is the Blank control:

*Control lysates are supplied as a control reagent - not an absolute quantitation measure. A 3 - 4 point lysate dilution series is sufficient for this purpose.

Note: the extent of the dilution series appropriate for your samples needs to be determined empirically. Further dilutions than those shown below may be required.



Sample Preparation

A cell density that yields 10,000 – 40,000 cells/well is suitable for the analysis of many cell lines. The lysis buffer volume should be adjusted so that lysates are in the range of 100-500 µg/mL of protein.

Preparation of extracts from cell pellets: Collect non-adherent cells by centrifugation and resuspend at an appropriate density in RPMI containing 10% FBS. Typical centrifugation conditions for cells are 500 x g for 5 minutes at RT. Return cells to a 37°C incubator for 1 - 2 hours. For certain pathways, this can allow handling-mediated pathway activation to subside. This step is optional and depends on the activation status of your cells following re-suspension. At the completion of the cell treatment, harvest cells by centrifugation and lyse with 1X Cell Extraction Buffer PTR.* Alternatively, in the absence of centrifugation cells may be lysed directly with a 20% volume of 5X Cell Extraction/Enhancer Buffer PTR (e.g. for 80 µL of cells, use 20 µL of 5X Cell Extraction/Enhancer Buffer PTR). Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.
*For best results, we recommend centrifugation and lysis of cells with 1X Cell Extraction Buffer PTR. Matrix effects may be observed in different cell media using the direct lysis approach (alternative protocol).
Preparation of extracts from adherent cells by direct lysis (alternative protocol): Remove growth media and rinse adherent cells 2 times in PBS. Solubilize the cells by addition of chilled 1X Cell Extraction Buffer PTR directly to the plate (for cells cultured in 96-well microplates, lyse the cells with 100 µL* of 1X Cell Extraction Buffer PTR).
*Lysis volume should be adjusted depending on the desired lysate concentration. Lysates in the range of 100 - 500 µg/mL protein are usually sufficient.
Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.
Preparation of extracts from tissue homogenates: Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended). Homogenize 100 to 200 mg of wet tissue in 500 µL – 1 mL of chilled 1X Cell Extraction Buffer PTR. For lower amounts of tissue adjust volumes accordingly. Incubate on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

Plate Preparation

The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
For each assay performed, a minimum of two wells must be used as the zero control.
For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
Differences in well absorbance or "edge effects" have not been observed with this assay.

Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.
We recommend that you assay all standards, controls and samples in duplicate

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.
3. Add 50 µL of all sample or standard to appropriate wells.
4. Add 50 µL of the Antibody Cocktail to each well.
5. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.
6. Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.
7. Add 100 µL of TMB Development Solution to each well and incubate for 15 minutes in the dark on a plate shaker set to 400 rpm.
8. Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.
9. Alternative to 7 – 8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode	Kinetic
Wavelength	600 nm
Time	up to 15 min
Interval	20 sec - 1 min
Shake	Shake between readings

Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips: www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus

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